Journal of Computing, Science &Technology
https://focjournal.unidel.edu.ng/
editorjcst@unidel.edu.ng
ISSN:.....Print
ISSN: ..... Online

Determination of Minimum Inhibitory Concentration of Clinical Isolates From a Hospital in South -South Nigeria

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Article Info

Date Received: 30-01-2024
Date Accepted: 24-03-2024

Keywords:
Agar dilution, cumulative sensitive profile, clinical bacterial isolates.

ABSTRACT

Infections caused by microorganisms have become a significant public health concern in Nigeria, leading to increased morbidity and mortality rates. This study was designed to evaluate the in-vitro activities of three commonly prescribed quinolone and cephalosporin agents against clinical bacterial isolates from Central Hospital Warri. The minimum inhibitory concentrations (MIC) were investigated using the agar dilution method. The invitro activity of quinolones- pefloxacin, ciprofloxacin, ofloxacin were compared with 3 other agents namely ceftazidime, cefuroxime, and gentamicin. Pefloxacin showed greater activity followed by ofloxacin and ciprofloxacin. Overall, the MIC_{90} values for the quinolones, ciprofloxacin, pefloxacin, and ofloxacin were below 2 mg/l. Pefloxacin and ofloxacin inhibited 90% of isolates of Escherichia coli, Klebsiella sp, Staphylococcus aureus and Proteus sp at values <0.6mg/l. The MIC_{90} value for ciprofloxacin was higher with 1.76 mg/l as MIC_{90}, for Klebsiella sp in this study. Against Pseudomonas aeruginosa, ofloxacin had MIC_{90} values of 2.75 mg/l. The MIC_{90} for Staphylococcus aureus was 0.5, 0.7 and 1.2 mg/l respectively for pefloxacin, ofloxacin and ciprofloxacin. Pefloxacin was therefore adjudged the most active of the quinolone compounds in this study. However, the in-vitro potency of pefloxacin was equivalent to that of ofloxacin which could be prescribed as an alternative, but greater than those of cefuroxime, ceftazidime, and gentamicin. It is therefore pertinent to state that medical laboratories should periodically review their antibiotic usage to curb the resistant issues among organisms.

1.0 INTRODUCTION

Bacterial infections are linked to an increased risk of morbidity and mortality. Antimicrobial susceptibility testing is becoming more important due to the rising resistance of organisms to existing antimicrobial treatments. It is performed to identify which antimicrobial regimen is specifically effective for individual patients under laboratory conditions [11]. The inefficacy of current medical therapies in addressing bacterial infections necessitates a proactive search for therapeutic approaches and the meticulous selection of antibiotics, considering a range of parameters, notably microbiological aspects. Minimal inhibitory concentration (MIC) defines in vitro levels of susceptibility or resistance of specific bacterial strains to applied antibiotic [6]. The emergence and persistence of antimicrobial resistance among clinical isolates pose significant challenges in health care settings. This study seeks to provide insights.

of the minimum inhibitory concentration of clinical isolates against routinely used drugs for antimicrobial susceptibility testing in Central Hospital Warri, Delta State Nigeria.

2.0 METHODOLOGY

2.1 Setting

This laboratory-based study was carried out at the Department of Laboratory Services, Central Hospital Warri, Delta State, Nigeria.

2.2 Isolates:

One hundred and eighteen (118) isolates recovered from a clinical sample were used. The isolates were identified and characterised using standard bacteriological techniques [2].

2.3 Antimicrobial agents

The following commonly prescribed antimicrobial agents were tested. These include three (3) fluroquinolones (ciprofloxacin, ofloxacin and pefloxacin); Two (2) cephalosporin (cefuroxime and ceftazidime); and one(1) aminoglycoside (gentamicin). The antimicrobial agents were dissolved into sterile distilled water as according to

manufactures instructions. A stock solution was prepared aseptically. Antimicrobial agents were diluted from the stock solutions to make as series of two-fold dilutions.

The minimum inhibitory concentration (MIC) of the antibacterial agents were determined using the agar dilution method [14] and as checked in accordance to the Clinical Laboratory Standard Institute antimicrobial susceptibility testing standards guidelines (Wayne, 2015). A range of dilutions of antibacterial agents at 20 times the final concentration required in the agar was made in sterile distilled water. One millilitre of the appropriate antimicrobial concentration was added to 19ml molten agar in glass universal bottle and mixed gently avoiding the formation bubbles and poured into a suitably labelled Petridish. A growth control plates were to gel and then dried in a non-humidified incubator at 35 – 37°C until all surface moisture had been removed. The plated were incubated with 0.02 ml of an overnight culture of the bacterial isolates diluted1/100 (containing 10^5 cells/ml) using sterile micropipette tips and incubated at 37°C. The MIC of the antimicrobial agents tested were defined as the lowest concentration of the agent which inhibited visible growth of the test organism after 18-24 hrs incubation at 37°C [1].

3.0 RESULTS AND DISCUSSION

The present study was carried out to determine the MICs of six commonly prescribed antimicrobial drugs. The Overall the MIC_{90} values for the quinolone was below 2 mg/dl. These findings are like those of Gupta *et al.*, 2020[4]. The MIC_{90} values of the quinolones observed in this study were less than 2 mg/l except for *Pseudomonas aeruginosa*, which had an MIC_{90} value of 2.75 mg/l for ofloxacin. The minimum inhibitory concentration (MIC) which underlies all antimicrobial susceptibility testing is largely ignored in the decision-making process of optimal drug selection[8].

Table1 shows the cumulative minimum inhibitory concentration of isolates to the tested fluoroquinolones.

Table 1.MIC value of fluoroquinolones among the bacterial strains tested.

Organisms	*Antibacterial agents*	*Number of Isolates Inhibited by (mg/l)*											
		0.04	0.25	0.5	1	2	4	8	*16*	*32*	*64*	*128*	*226*
	Ciprofloxacin	-	4	8	7	10	-	1	3	1	3	-	-
Staphylococcus aureus (37)*	Ofloxacin	3	6	12	7	2	4	-	-	-	-		-
	Pefloxacin	3	15	8	11	-	-	-	-	-	-		-
	Ciprofloxacin	-	-	-	-	-	1	-	-	-	-	-	-
*Streptococcus sp (1)**	Ofloxacin	-	-	-	-	1	-	-	-	-	-	-	-
	Pefloxacin	-	-	1	-	-	-	-	-	-			-
	Ciprofloxacin	-	-	10	7	6	4	2	-	2	1	2	-
Klebstella Sp (33)*	Ofloxacin	2	7	9	9	-	-	-	4	-	-	-	-
	Pefloxacin	-	2	11	13	7	-	-	-	-	-	-	-
	Ciprofloxacin	2	-	10	5	3	-	-	-	1	3	1	-
Escherichia coli (26)	Ofloxacin	4	6	9	2	2	-	-	-	1	2	-	-
	Pefloxacin	6	5	6	6	3	-	-	-	-	-	-	-
	Ciprofloxacin	-	-	4	2	-	2	-	-	-	2	-	-
Proteus sp (10)*	Ofloxacin	1	3	2	1	-	-	-	1	1	-	1	-
	Pefloxacin	-	1	2	5	1	3	-	-	-	-	-	-
	Ciprofloxacin	1	-	-	3	3	1	-	-	-	-	-	-
Pseudomonas ae ruginosa (11)*	Ofloxacin	-	-	1	1	1	1	2	-	1	-	1	-
	Pefloxacin	-	-	2	5	-	3	-	-	-	-	-	-

Table 2: MIC value of cephalosporins among the bacterial strains tested.

Organisms	Antibacterial agents	Number of Isolates Inhibited by (mg/l)													
		0.05	0.2	0.5	1	1.25	2.5	5	7.5	10	15	30	50	100	150
Staphylococcus aureus (37)*	Cefuroxime	-	2	-	7	2	8	4	3	2	-	-	-	2	4
	Ceftazidime	-	-	2	6	2	5	7	6	1	2	-	3	2	1
*Streptococcus sp (1)**	Cefuroxime	-	-	-	-	-	1	-	-	-	-	-	-	-	-
	Ceftazidime	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Klebsiella Sp (33)*	Cefuroxime	-	-	-	3	-	7	9	2	4	-	-	-	4	4
	Ceftazidime	-	-	-	7	1	5	6	10	-	-	-	-	2	1
Escherichia coli (26) *	Cefuroxime	-	-	-	-	-	13	5	2	1	-	-	-	1	3
	Ceftazidime	-	-	-	9	3	2	9	-	-	-	-	2	1	-
Proteus sp (10) *	Cefuroxime	-	-	4	5	-	-	1	-	-	-	-	-	-	-
	Ceftazidime	-	-	-	1	2	1	2	1	-	-	-	1	2	-
Pseudomonas aeruginosa (11) *	Cefuroxime	-	-	1	6	2	1	1	-	-	-	-	-	-	-
	Ceftazidime	-	-	-	1	2	1	4	-	-	-	-	2	-	1

*= Number tested in parenthesis

The MIC range of ciprofloxacin was between 0.25 - 8 mg/l for susceptible organisms. For *Staphylococcus aureus*, *Escherichia coli* 0.04 - 2 mg/l, P*roteus* sp. 0.5-4 mg/l, *Pseudomonas aeruginosa* 0.04 - 2 mg/l and Klebsiella sp. 0.5 8 mg/l, while S*treptococcus* sp was susceptible at concentration of 4 mg/l. MIC range for ofloxacin was 0.04 - 128 mg/l, while the killing ranges were observed, for susceptible organism were between 0.025 - 8mg/l, while resistant organisms had range of 32-128 mg/l The MIC for individual species were *Staphylococcus aureus* 0.04-4 mg/l, *Escherichia coli* 0.5 - 8 mg/l, while *Klebsiella* sp. and *Proteus* species had MIC range of 0.04 - 1 mg/l. Two *isolates Pseudomonas aeruginosa* had an MIC value of 128 mg/l, *Streptococcus* sp was susceptible at concentration of 2 mg/l. The overall MIC for pefloxacin ranged from 0.25 - 8 mg/l. The MIC ranges for individual species were *Staphylococcus aureus* 0.25 4 mg/l, *Klebsiella* sp. 0.25 - 2 mg/l. *Escherichia coli* and *Proteus* sp. had the same MIC range of 0.04 - 4 mg/l while *Pseudomonas aeruginosa* had arrange of 0.5-8mg/l, *Streptococcus* sp was susceptible at concentrationof0.5 mg/l.

Table 2 shows the MIC values for the tested cephalosporins in this study. The MIC values of cefuroxime against isolates ranged from 0.2- 10 mg/l. For individual species MIC ranges were *Staphylococcus aureus* 0.2 -1.0mg/l, *Streptococcus* sp was susceptible at concentration of 2.5 mg/l. *Escherichia coli* 2.5 - 10 mg/l, *Pseudomonas aeruginosa* 0.5 - 7.5 mg/l and *Proteus* sp 1-5 mg/l. The MIC values ceftazidime susceptible organisms ranged from 1 - 15mg/l. While resistance above 50 mg/l was found in eleven isolates of Pseudomonas aeruginosa. The MIC

Table 3: MIC value of Gentamicin Among *the* Bacterial Strains Tested.

Organisms	Number of Isolates Inhibited by (mg/l)													
	0.05	0.2	0.5	1	1.25	2.5	5	7.5	10	15	30	50	75	150
Staphylococcus aureus (37)*	-	-	-	-	-	14	11	5	2	-	3	1	-	-
*Streptococcus sp (1)**	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Klebsiella Sp (33)*	-	-	-	3	-	8	4	2	4	3	8	1	-	-
Escherichia coli (26)	-	3	1	4	-	5	2	1	-	1	5	3	1	-
Proteus sp (10)*	-	-	-	-	-	1	4	2	-	-	2	1	-	-
Pseudomonas aeruginosa (11)*	-	-	4	4	-	2	1	-	-	-	-	-	-	-

*= Number tested in parenthesis

ranges for individual species were *Staphylococcus aureus* 0.5 - 15 mg/l, Klebsiella sp. and *Proteus* sp. had the same MIC range of 1 - 7.5 mg/l with *Escherichia coli* and *Pseudomonas aeruginosa* showing the same range of 1-5mg/l. Indicating that ceftazidime has a better anti Pseudomonal and Proteus sp. activity. *Streptococcus* sp was susceptible at concentration of7.5 mg/l.

Table 3 shows the MIC value of gentamicin. The gentamicin MIC ranged from 0.2 - 30mg/l. The MIC ranges for individual species were 2.5 - 10 mg/l for *Staphylococcus aureus, Escherichia coli* 0.2 - 12.5 mg/l Proteus sp. 2.5 - 7.5 mg/l, *Pseudomonas aeruginosa* 0.5-5 mg/l and *Klebsiella* sp 2.5-5 mg/l.

Table 4 shows MIC values of the agents required to inhibit 90% of the strains. The MIC required to inhibit 90% of *Staphylococcus aureus* isolates were pefloxacin 0.5 mg/l, ofloxacin0.7 mg/l, ciprofloxacin 1.2 mg/l. For klebsiella sp. pefloxacin inhibited 90% of isolates at 0.6mg/l, ofloxacin,0.5 mg/l, and ciprofloxacin1.76 mg/l. *Escherichia coli* was inhibited by0.25 mg/l, pefloxacin, ofloxacin 0.5mg/l and ciprofloxacin 0.7 mg/l. pefloxacin 0.32 mg/l, ofloxacin 0.36 mg/l and ciprofloxacin 1.25 mg/l inhibited isolates of *Proteus* sp. While 90% of *Pseudomonas aeruginosa* isolates were inhibited at a concentration of 0.5mg/l for pefloxacin, ofloxacin 2.75 mg/l, and ciprofloxacin1.0 mg/l.

The emergence of organisms that are resistant to the majority classes of antimicrobial agents has become a serious public health concern. Due to the selection pressure created by misuse of antibiotics, the emergence of pathogenic bacteria species with antimicrobial resistance (AMR) is an expected evolutionary process [12]. Antimicrobial drug resistance is a leading cause of treatment failure, prolonged hospitalisation and even death

Table 4: MIC_{50} and MIC_{90} Value for the Tested Antimicrobial Agent

Organisms	Antibiotic	Range	MIC mg/l 50%	MIC mg/l 90%
Staphylococcus aureus	Pefloxacin	0.25 – 1	0.25	0.5
	Ofloxacin	0.04 – 4	0.4	0.7
	Ciprofloxacin	0.25 – 8	0.6	1.2
	Cefuroxime	0.5 – 1.5	2.0	4.0
	Ceftazidime	0.2 – 10	1.5	2.7
	Gentamicin	2.5 – 10	2.3	4.0
Klebsiella Spp	Pefloxacin	0.25 – 2	0.3	0.6
	Ofloxacin	0.04 – 1	0.2	0.5
	Ciprofloxacin	1 – 8	0.98	1.76
	Cefuroxime	1 – 7.5	2.0	4.0
	Ceftazidime	1 – 10	2.4	4.3
	Gentamicin	1 – 12.5	3.2	5.7
Escherichia coli	Pefloxacin	0.04 – 2	0.06	0.25
	Ofloxacin	0.04 – 2	0.26	0.50
	Ciprofloxacin	0.04 – 2	0.4	0.70
	Cefuroxime	1 – 5	1.0	2.5
	Ceftazidime	2.5 – 10	1.9	3.5
	Gentamicin	0.2 – 12.5	1.4	2.5
Proteus spp	Pefloxacin	0.04 – 1	0.20	0.32
	Ofloxacin	0.04 – 1	0.20	0.36
	Ciprofloxacin	0.5 – 4	0.75	1.35
	Cefuroxime	1 – 7.5	1.68	3.0
	Ceftazidime	1 – 5	0.6	1.08
	Gentamicin	2.5 – 7.5	2.6	4.8
Pseudomonas aeruginosa	Pefloxacin	0.5 – 4	0.25	0.5
	Ofloxacin	2.5 – 4	1.5	2.75
	Ciprofloxacin	0.04 – 2	0.5	1.0
	Cefuroxime	1.5	1.6	2.0
	Ceftazidime	0.5 – 7.5	0.86	1.55
	Gentamicin	0.5 – 5	0.72	1.31

[7]. Drugs such as fluorinated quinolones appear to have a place in the treatment of commonly acquired infection that are resistant to previously established agents[10]. Although fluroquinolones have proved effective in various types of infection, they should seldom be the drug of choice[9]. Hence when treatment with fluroquinolone is considered for specific infections certain guidelines should be followed. First, a quinolone should be used when alternative antibiotics are more toxic or less efficacious for specific infections (King *et al.,* 2000). Secondly, use of a quinolone can be considered when a patient has a history of a severe allergy or adverse effect to one or the usually indicated antibiotics. Thirdly, a quinolone should be chosen when an infection is caused by multiple resistant bacteria and usually necessitates treatment with two or more antibiotics. Fourth, a quinolone can be selected when use of a parentally administered agent treatment of resistant bacteria can be avoided. Fifth, changing treatment of a quinolone can be considered for completion of parental therapy as an outpatient. Quinolone should be prescribed when it is clearly the preferred drug of choice for a particular infection[5].The use of these drugs on a much more regular basis would greatly increase the selective pressure for the rise in resistance compared to a situation where they are used as agents of second or third choice[3].

4.0 CONCLUSION

This study has shown that agents such as fluroquinolones offer a viable alternative to older agents such as penicillin and ampicillin in treatment of infections. It is therefore advocated that the microbiology laboratory should frequently review its cumulative sensitivity profile for antibiotics to advise Clinicians on any changing institutional trends so that empirical usage of antibiotics can be adjusted accordingly. A State/National antibiotic resistant programme should be carried out since the result of such programme will aid in accumulating epidemiologic data on resistance of medically important bacteria in Nigeria.

Competing Interests There are no competing interests.

Financial Support and Sponsorship: - Nil.

5.0 ACKNOWLEDGMENT

Our debt of gratitude to the patients who voluntarily consented to participating in this study; Also, to the Medical Laboratory Staff of Central Hospital Warri, Delta State for their technical support.

REFERENCES

[1] Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, *48*(suppl_1). https://doi.org/10.1093/jac/48.suppl_1.5

[2] Cowan, S. T., & Steel, K. J. (1994). *Cowan and Steel's Manual for the Identification of Medical Bacteria.* Cambridge University Press.

[3] Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews: MMBR*, *74*(3), 417–433. https://doi.org/10.1128/MMBR.00016-10

[4] Gupta, V., Pal, K., Bhagat, A., Goel, A., & Chander, J. (2020). Quinolone Susceptibility in Salmonella Isolates Based on Minimum Inhibitory Concentration Determination. *Journal of Laboratory Physicians*, *12*(04), 263–267. https://doi.org/10.1055/s-0040-1721163

[5] King, D. E., Malone, R., & Lilley, S. H. (2000). New classification and update on the quinolone antibiotics. *American Family Physician*, *61*(9), 2741–2748.

[6] Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. *Pathogens*, *10*(2), 165. https://doi.org/10.3390/pathogens10020165

[7] Leopold, S. J., van Leth, F., Tarekegn, H., & Schultsz, C. (2014). Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: a systematic review. *The Journal of Antimicrobial Chemotherapy*, *69*(9), 2337–2353. https://doi.org/10.1093/jac/dku176

[8] Lowman, W. (2018). Minimum inhibitory concentration-guided antimicrobial therapy – The achilles heel in the antimicrobial stewardship agenda. In *South African Medical Journal* (Vol. 108, Issue 9). https://doi.org/10.7196/SAMJ. 2018.v108i9.13285

[9] Oni, A. A., Bakare, R. A., Arowojolu, A. O., Kehinde Toki, R. A., & Fasina, N. A. (2001). Comparative in-vitro activities of commonly available quinolones and other antibiotics on bacterial isolates in Ibadan, Nigeria. *African Journal of Medicine and Medical Sciences*, *30*(1–2), 35–37.

[10] Pham, T. D. M., Ziora, Z. M., & Blaskovich, M. A. T. (2019). Quinolone antibiotics. *MedChemComm*, *10*(10), 1719–1739. https://doi.org/10.1039/c9md00120d

[11] Schofield, C. B. (2012). Updating antimicrobial susceptibility testing: methods. *Clinical Laboratory Science: Journal of the American Society for Medical Technology*, *25*(4). https://doi.org/10.29074/ascls.25.4.233

[12] Tenover, F. C. (2009). Antibiotic Susceptibility Testing. *Encyclopedia of Microbiology*, 67–77. https://doi.org/10.1016/B978-012373944-5.00239-X

[13] Wayne, P. A. (2015). *M02-A11: Performance standards for antimicrobial disk susceptibility tests; Approved Standard* (Vol. 32, Issue 1)

[14] Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, *3*(2), 163–175. https://doi.org/10.1038/nprot.2007.52